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ORIGINAL ARTICLE

Testosterone stimulates extra-hepatic but not hepatic fat oxidation (Fox): comparison of oral and transdermal testosterone administration in hypopituitary men

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Summary

Background Fat mass is increased in hypogonadal men and the changes are reversed by testosterone replacement. Testosterone administration enhances whole body fat oxidation (Fox). Fat is oxidized in the liver and in extra-hepatic tissues.

Objective To determine whether the stimulation of Fox by testosterone arises primarily from the liver or from extra-hepatic tissues.

Design/patients This was an open-label cross-over study. Thirteen men with hypopituitarism (age 53·1 ± 4·1 years) with both growth hormone (GH) and testosterone deficiency were studied sequentially after 2 weeks of treatment with transdermal testosterone (5 mg), no treatment, and stepwise incremental doses of oral crystalline testosterone (10, 20, 40 and 80 mg) in the absence of GH replacement.

Measurements Serum testosterone, IGF-I, metabolic effects [resting energy expenditure (REE) and Fox], SHBG, and thyroid binding globulin (TBG) as markers of excessive hepatic androgen exposure, were measured at the end of each treatment period.

Results When compared to the no-treatment phase, mean blood testosterone levels rose into the physiological range after transdermal testosterone delivery but did not significantly change after 10, 20, 40 or 80 mg oral testosterone treatment. Blood SHBG and TBG fell significantly with 80 mg oral testosterone dose but were unaffected by any other testosterone treatment. Fox increased significantly with transdermal but not with any dose of oral testosterone. Mean plasma IGF-I and REE were unaffected by testosterone, regardless of the route or dose.

Conclusions Short-term testosterone administration does not stimulate hepatic fat oxidation but enhances whole body fat oxidation by acting on extra-hepatic tissues.

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Introduction

Testosterone is a major anabolic hormone which plays an important role in regulating body fat.[1,2] A mechanism by which testosterone regulates fat mass is the stimulation of fat oxidation (Fox).[3–5] Fat is oxidized in the liver and in extra-hepatic tissues, such as skeletal muscle. The liver is a major metabolic organ that is sensitive to sex steroids. Oestrogen, when administered orally, suppresses whole body Fox.[6–8] However, this does not occur with transdermal administration, indicating that the suppressive effect arises from a first pass effect of oestrogen on hepatic Fox.[6] The suppression of Fox by oestrogen administered orally translates into a significant increase in fat mass over time.[6]

We recently reported that intramuscular administration of testosterone enhances Fox in men with hypopituitarism with or without GH replacement.[3] However, whether this arises from stimulation of Fox from the liver is not known. To determine if the site where testosterone stimulates Fox is hepatic or extra-hepatic, we compared the effect of crystalline, unconjugated testosterone administered via the oral route with a standard transdermal testosterone replacement dose. Oral delivery of testosterone exposes the liver to high portal levels of testosterone which undergoes first-pass hepatic metabolism preventing its appearance in the systemic circulation. In contrast, testosterone administered transdermally enters the systemic circulation directly bypassing hepatic metabolism. This was a dose-finding study to determine the dose of orally delivered testosterone which would cause hepatic androgenic effect. Previous studies have indicated that oral testosterone dose in excess of 200 mg/day is required to increase testosterone levels in peripheral blood.[9–11] In this study we used oral doses of testosterone designed to increase portal but not peripheral blood testosterone concentrations.

Table 1. Characteristics of hypogonadal GH-deficient subjects

Subject number	Age, year	BMI	Diagnosis	Treatment	Hormone replacement
1	36	26·6	Craniopharyngioma	S	A, T, G
2	28	35·8	Idiopathic hypopituitarism	nil	A, T, G, D
3	66	23·6	Rathke's cyst	S	A, T, G, D
4	64	22·1	Pituitary macroadenoma	S	T, G
5	52	29·8	Rathke's cyst	S	A, T, G
6	54	30·0	Pituitary macroadenoma	S	A, T, G,
7	47	23·7	Pituitary macroadenoma	S	A, T, G, D
8	52	29·3	Pituitary macroadenoma	S	A, T, G
9	35	24·8	Pituitary macroadenoma	S	A, T, G, D
10	65	27·9	Pituitary macroadenoma	M	T, G
11	73	26·7	Pituitary macroadenoma	S	A, T, G
12	73	27·6	Pituitary macroadenoma	S	A, T, G
13	45	38·8	Congenital hypopituitarism	nil	T, G

BMI, body mass index; S, surgery; M, medication; A, adrenal replacement; T, thyroid replacement; G, gonadal replacement; D, deamino-8-arginine vasopressin.

Subjects and methods

Subjects

Thirteen hypopituitary men with androgen and GH deficiency were recruited from the Endocrine Outpatient Clinic, St Vincent's Hospital, Sydney, Australia. The clinical characteristics of the patients are shown in Table 1. The duration of hypopituitarism was at least 1 year. GH deficiency was established by the insulin tolerance test (a peak GH response to insulin-induced hypoglycaemia of < 3 μg/l[12]). None of subjects had ever received GH replacement. Secondary hypogonadism was confirmed by serum testosterone of < 6 nmol/l, and by low values of LH.

Subjects on testosterone replacement discontinued the treatment before commencement of the study. The washout period was at least 2 weeks for transdermal testosterone, 6 weeks for intramuscular testosterone esters, and 6 months for testosterone implants. All subjects had thyroid hormone deficiency, which was replaced with standard doses of T4 for at least 1 year before the commencement of study. Ten out of 13 subjects received standard cortisol replacement for adrenal deficiency prior to and throughout the study. The doses of replacement were unchanged throughout the study. GH deficiency was not replaced. All subjects were instructed to follow their usual diet and physical activity as well as continuing their usual medications or supplements throughout the study.

St. Vincent's Hospital Human Research Ethics Committee approved the study. All study participants gave written informed consent. The study was registered with the Australian and New Zealand Clinical Trials Registry (ACTRN12605000482662).

Study design

This was an open-label, fixed sequence cross-over study. Subjects were entered into the study only after an appropriate period of testosterone washout. They were then assigned sequentially to transdermal testosterone (Androderm patches, Mayne Pharma Ltd, Australia; 5 mg), a control no-treatment phase (2 weeks), followed by stepwise incremental doses of oral crystalline testosterone at 10, 20, 40 and 80 mg daily (divided in three doses taken every 8 h), with each treatment period of 2 weeks duration (Fig. 1). At baseline and the end of each treatment period, indirect calorimetry was performed and blood samples were taken for measurement of testosterone, IGF-I, SHBG, thyroid binding globulin (TBG), HDL and total cholesterol.

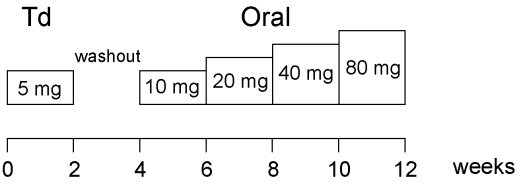

Fig. 1 Study design. Testosterone was administered as daily transdermal (Td) Androderm 5 mg patches or as oral crystalline testosterone in stepwise incremental doses (10, 20, 40 and 80 mg/day), with each treatment period lasting for 2 weeks.

Oral testosterone was prepared by Fresh Therapeutics (Sydney, Australia) as capsules filled with crystalline testosterone USP without excipients. The dose of oral testosterone was based on known pharmacokinetics[9–11,13,14] and designed to increase portal but not peripheral blood testosterone concentrations with an androgenic effect on hepatic function as indicated by effects on SHBG and TBG.[15–17] Because of the absence of prior data as to what dose of oral testosterone induces a pharmacological effect, we used a dose finding study which committed us to a stepwise sequential design. Previous studies have indicated that oral testosterone doses in excess of 200 mg/day are required to increase testosterone levels in peripheral blood.[9–11] Compared with the endogenous testosterone production rates of 5–10 mg/day,[18–20] such doses are clearly pharmacological as they induce marked changes in hepatic function.[21,22] For this study, much smaller doses were used starting at 10 mg and increasing in a stepwise manner aiming to increase portal but not peripheral testosterone concentrations while also manifesting androgenic effects on hepatic function (secretion of SHBG and TBG).

Indirect calorimetry

Subjects were studied in the Clinical Research Facility, Garvan Institute of Medical Research.

Resting energy expenditure (REE) and substrate metabolism (fat and carbohydrate oxidation) were quantified after an overnight fast by indirect calorimetry (Deltatrac Metabolic Monitor, Datex Instrumentarium Corp., Helsinki, Finland), which was calibrated against standard gases before each study. Subjects were placed on a bed and allowed to rest for at least 30 min. A clear plastic hood was placed loosely over the subject's head and shoulders for a 20-min period. O_2 consumption and CO_2 production was measured and REE, Fox and carbohydrate oxidation (Cox) estimated using weight-based equations adjusted from Ferrannini.[23]

$$\text{REE} = ((3{\cdot}91 \times \text{VO}_2/1000) + (1{\cdot}1 \times \text{VCO}_2/1000) - (3{\cdot}34 \times 0{\cdot}14 \times \text{weight}/1440)) \times 1440;$$

$$\text{Fox} = ((1{\cdot}67 \times \text{VO}_2/1000) - (1{\cdot}67 \times \text{VCO}_2/1000) - (1{\cdot}92 \times 0{\cdot}14 \times \text{weight}/1440)) \times 1000;$$

$$\text{Cox} = ((4{\cdot}55 \times \text{VCO}_2/1000) - (3{\cdot}21 \times \text{VO}_2/1000) - (2{\cdot}87 \times 0{\cdot}14 \times \text{weight}/1440)) \times 1000.$$

VO_2 represents oxygen consumption and VCO_2 represents carbon dioxide production in litres per minute. REE are expressed as kcal/d, Fox and Cox are expressed in mg/min. The mean intra-subject coefficients of variation (CV) for REE and Fox at our institution are 4·2% and 4%, respectively.[6]

Assays

Serum testosterone, SHBG, and PSA (Prostate Specific Antigen) were measured by RIA using commercial assays (Immulite 2000, Siemens Medical Solution Diagnostics, Los Angeles, CA). The inter-assay CVs for testosterone at 3·6 and 23 nmol/l were 9·3% and 9·0%, respectively. The CVs for SHBG at 5·3 and 86·2 nmol/l were 5·0% and 7·5%, respectively. The CVs for PSA was 7·6% at 0·33 μg/l and 5·0% at 10 μg/l. Serum TBG levels were measured by chemiluminescent immunoassay (Immulite 2000, Siemens Medical Solution Diagnostics). The CVs for TBG at 7·08 and 35·0 mg/l were 17·8% and 9·9%, respectively. Serum IGF-I level was measured by RIA after acid ethanol extraction as previously described.[3,7,8] The CVs for IGF-I were 8·3% at 14·7 nmol/l and 7·4% at 28·6 nmol/l. HDL and total cholesterol were measured using Enzyme Colorimetric method (E-170 kit, Roche Diagnostics, Indianapolis, IN). The CV for total cholesterol was 1·7% at 5·4 mmol/l. The CVs for HDL-cholesterol were 1·3% at 0·82 mmol/l and 1·2% at 1·64 mmol/l.

Statistical analysis

All data are expressed as mean ± SEM. Treatment effects were analysed by repeated measures ANOVA followed by paired *T*-tests with significance ($P < 0{\cdot}05$) determined after Bonferroni's correction. Statistical analysis was undertaken using the statistical software packages STATVIEW 4·5 PPC (Abacus Concepts, Inc, Berkeley, CA).

Results

The mean age of the subjects was 53·1 years (range 28–73 years), with mean weight 82·6 ± 3·7 kg and mean BMI 28·2 ± 1·3 kg/m^2 (mean ± SEM). PSA levels for all subjects ranged from 0·13 to 4·4 μg/l.

The mean testosterone level at screening was 4·7 ± 0·7 nmol/l. After transdermal administration, the mean testosterone level (16·6 ± 3·1 nmol/l) rose into the adult normal range (12–36 nmol/l). The mean testosterone level fell to pre-treatment baseline levels of 3·9 ± 0·7 nmol/l 2 weeks after terminating transdermal testosterone treatment during the no-treatment phase. The mean peripheral blood testosterone concentration did not rise significantly with oral administration of 10, 20, 40 or 80 mg per day of crystalline testosterone (Fig. 2a). The mean testosterone level after transdermal administration was significantly higher than those observed at all phases of oral treatment ($P < 0{\cdot}01$; Fig. 2a).

Mean SHBG level after transdermal testosterone administration was not significantly different from that observed after the no-treatment phase. After the oral treatment phase, mean SHBG levels observed following administration of 10, 20, and 40 mg/day of testosterone were not significantly different from the no-treatment phase. However, the mean SHBG level was significantly lower after 80 mg/day of oral testosterone than that observed after the no-treatment phase ($P < 0{\cdot}01$; Fig. 2b). Mean TBG levels after transdermal or oral treatment with 10, 20, and 40 mg/day of testosterone administration were not significantly different from that observed after the no-treatment phase. However, TBG level was significantly lower with 80 mg/day of oral testosterone compared to that observed after the no-treatment phase ($P < 0{\cdot}05$; Fig. 2c).

Mean REE did not differ significantly between the no-treatment, transdermal or oral testosterone phases. There was no significant difference in REE between transdermal and any oral doses of testosterone administration (Table 2). When compared to the no-treatment phase, Fox was significantly higher following transdermal testosterone administration ($P < 0{\cdot}01$; Fig. 3). There was no significant difference in Fox between the no-treatment phase and those observed after any dose of oral testosterone administration. Mean Cox was not significantly different between any treatment phases (data not shown).

The mean concentration of total cholesterol was not significantly different between the no-treatment phase and transdermal or oral testosterone administration, or between transdermal and any oral doses of testosterone administration (Table 2). However when compared to the no-treatment phase, there was a trend towards a lower total cholesterol level after treatment with the 80 mg oral testosterone dose although this did not reach statistical significance ($P = 0{\cdot}055$). Mean serum HDL-cholesterol levels were not significantly different between no-treatment phase and that observed after transdermal and oral testosterone administration, or between transdermal and any oral doses of testosterone administration (Table 2).

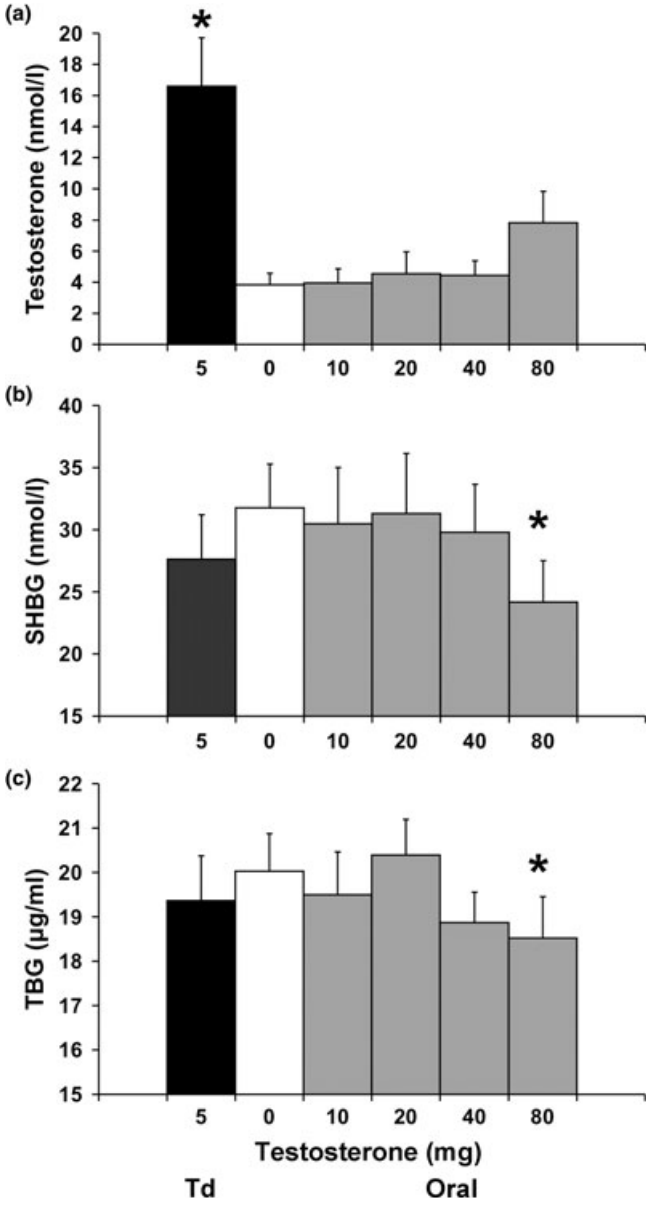

Fig. 2 (a) Serum testosterone levels (b) SHBG and (c) TBG serum levels in hypogonadal GH-deficient men following treatment with transdermal testosterone (Td, Androderm 5 mg) and incremental doses of oral crystalline testosterone. Data are presented as means ± SEM. $^*P < 0{\cdot}05$ compared to the no-treatment phase.

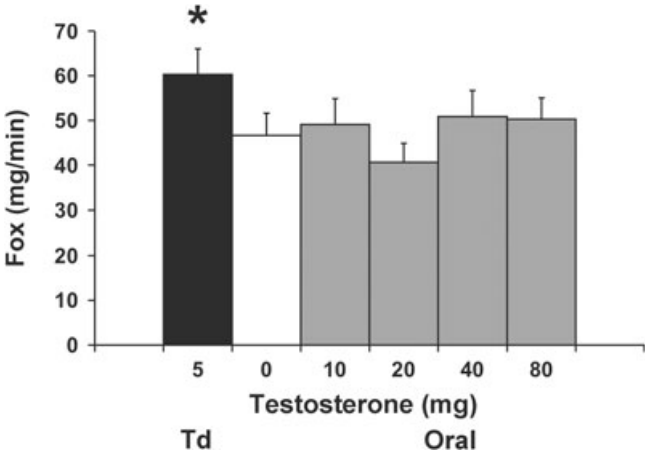

Fig. 3 Whole body fat oxidation rates in hypogonadal GH-deficient men following treatment with transdermal testosterone (Td, Androderm 5 mg) and incremental doses of oral crystalline testosterone. Data are presented as means ± SEM. $^*P < 0{\cdot}05$ compared to the no-treatment phase.

There was no significant difference in mean serum IGF-I concentration between transdermal testosterone, no treatment or oral testosterone phases (Table 2).

Discussion

This is the first study to investigate whether testosterone stimulates Fox in the liver. We studied whether the stimulation of whole body Fox was dependent on the route of testosterone administration in men with hypopituitarism. Transdermal delivery of a standard therapeutic dose normalized circulating blood levels of testosterone whereas oral delivery of 10–80 mg/day did not increase testosterone levels in peripheral blood. Whole body Fox was stimulated when testosterone was administered transdermally but not orally, even at the highest dose. The oral regimen induced a significant fall in SHBG and TBG concentrations at the highest dose and a slight fall in total cholesterol although the change did not reach statistical significance. In summary, this study demonstrates that oral testosterone at doses sufficient to induce physiological hepatic testosterone levels without increasing peripheral blood testosterone concentrations, does not stimulate Fox, which is however stimulated by transdermal administration that achieved physiological testosterone concentration in peripheral blood.

Table 2. Serum total (Total-C) and high density lipoprotein cholesterol (HDL-C), IGF-I concentrations, and resting energy expenditure (REE) in 13 hypogonadal subjects supplemented with either transdermal or increasing doses of oral crystalline testosterone for 2 weeks

		Transdermal‡	Oral‡			
	No treatment†	5 mg	10 mg	20 mg	40 mg	80 mg
Total-C (mmol/l)	5·53 ± 0·29	–0·14 ± 0·18	–0·21 ± 0·19	–0·03 ± 0·21	–0·21 ± 0·15	–0·39 ± 0·15
HDL-C (mmol/l)	1·36 ± 0·13	–0·03 ± 0·09	–0·11 ± 0·08	–0·05 ± 0·06	–0·03 ± 0·06	–0·04 ± 0·03
IGF-I (nmol/l)	12 ± 1·4	0·09 ± 0·41	–0·25 ± 0·22	–0·95 ± 0·45	–0·56 ± 0·39	0·82 ± 0·63
REE (kcal/day)	1508 ± 43	62·8 ± 37	87·2 ± 27	–17·0 ± 48	30·2 ± 32	58·3 ± 64

†Mean (± SEM) measured during the no treatment phase.
‡Values expressed as change from those observed during the no treatment phase.

There is strong evidence that testosterone regulates fat mass in addition to well-known protein anabolic effects.[2] Fat mass is increased in hypogonadal men compared to aged matched healthy men[24,25] and the changes are reversed by testosterone replacement.[24–29] The effect of testosterone on body fat in hypogonadal men is similar when administered by the transdermal route. A fall in fat mass occurs by 6 months and the reduction is maintained over 36 months of treatment.[24,29] Fat mass is also reduced in ageing men supplemented by testosterone treatment.[30–32] The mechanisms behind the reduction in fat mass by testosterone are complex and incompletely understood.

Testosterone can act directly on fat tissue to regulate lipid storage and number of adipocytes.[33] Testosterone inhibits lipid uptake by reducing lipoprotein lipase activity in adipocytes[34] and enhances adrenergic stimulation of lipolysis,[35] that is confirmed by human studies.[36] Testosterone also regulates the cellular mass of adipose tissue by inhibiting the differentiation of adipocyte precursor cells.[37] Lipid homeostasis can also be influenced by testosterone through effects on energy metabolism, specifically energy balance and lipid utilization. Testosterone deficiency is associated with decreased resting energy expenditure and lipid oxidation rate.[5] Because GH stimulates fat utilization and metabolism and testosterone enhances GH secretion,[38] it is possible that the effects of testosterone on fat mass may be GH mediated. However, our recent study demonstrated that testosterone stimulates whole body Fox in hypopituitary men with severe GH deficiency, providing strong evidence that androgens exert direct effect on fat metabolism.[3] While the liver is a major site of Fox, the process also occurs in many extra-hepatic tissues such as muscle, heart and skin.

The purpose of this study was to determine whether the site of testosterone-induced increase in whole body Fox is hepatic or extra-hepatic. Evidence that the metabolic function of the liver may be sex steroid regulated is based on studies in women in whom the effects of oestrogens administered via the oral and transdermal routes were compared. Oestrogens administered orally but not transdermally, suppressed whole body Fox.[6,7] That an effect did not occur with transdermal administration indicated that Fox was suppressed as a consequence of liver exposure to high levels of oestrogens in portal blood after oral administration.

Based on the findings of an inhibitory effect of oestrogens, we postulated that androgens stimulate hepatic Fox. We adopted a similar experimental paradigm to investigate the hepatic response by comparing the effects of testosterone administered via the oral and transdermal routes. Had the liver been androgen responsive, whole body Fox would have increased in parallel with the graded doses of oral testosterone. However, this was unaffected even by the highest dose which induced a hepatic pharmacological effect as indicated by a fall in SHBG and TBG concentrations.

The finding that Fox was increased by transdermal administration that normalized systemic testosterone levels in peripheral blood, and not by oral testosterone at the doses given, provides strong evidence that extra-hepatic and not hepatic Fox had been activated. This provides novel insights into the metabolic physiology of testosterone. The results indicate that Fox in hepatic and extra-hepatic tissues show different sensitivity to testosterone. Clinically the findings demonstrate that stimulation of lipid utilization that normally accompanies a testosterone replete state cannot be achieved by an oral testosterone dose that does not induce a hepatic pharmacological effect. Thus, not only do androgens and oestrogens exert opposite effects on Fox, their effects might be elaborated through different mechanisms, with the liver mediating the effects of oestrogens but not androgens.

It is possible that the hepatic contribution to whole body Fox from oral administration of testosterone may be obscured by a number of factors. First, the absence of a detectable stimulation of hepatic Fox may have arisen from the sensitivity of the technique. Second, the site of testosterone action in the liver may differ with regards to effects on androgenic markers and on fat metabolism. It is possible that the hepatocytes responsible for SHBG production and those most active in fat metabolism may not be optimally positioned relative to the portal blood supply (i.e. periportal *vs.* perivenous). However, this is unlikely because Braeuning *et al*. have reported that fatty acid metabolism occurs predominantly in the periportal region,[39] the area exposed to high concentration of hormones from the portal circulation. Finally, in men, testosterone is aromatized to oestrogens in many tissues.[40] Therefore, it is conceivable that the reported observation with testosterone may be weakened by aromatization to oestrogens. However this is unlikely because of strong evidence that aromatase activity is absent in adult liver.[41,42] Regardless of the mechanism, oral testosterone, in doses that resulted in pharmacological hepatic effect but no elevation in systemic concentration, failed to induce significant changes in Fox.

Increase in Fox by transdermal testosterone treatment was not paralleled by a change in resting energy expenditure. Since Cox did not change significantly, it is likely that a reduction in protein oxidation occurred. This is consistent with previous findings that intramuscular testosterone administration reduced protein oxidation in hypogonadal men.[3]

This study was not powered to detect an effect on total or HDL cholesterol levels, however high dose of oral testosterone showed a trend towards a reduction in total cholesterol levels. In the meta-analysis by Isidori *et al*.[2] testosterone decreased total cholesterol (especially in hypogonadal men) but no overall statistical difference was found for LDL and HDL cholesterol. However, a greater reduction in HDL was observed in men with higher baseline testosterone levels and in those treated with non-aromatizable androgens.[2] The data on the effect of oral testosterone administration are inconclusive with some studies reporting no change in serum total and HDL cholesterol levels[32,43,44] and other a fall in HDL cholesterol in healthy elderly men after 1-year treatment with testosterone undecanoate.[45] Blood lipid response to testosterone administration might differ between healthy elderly and hypogonadal men. The lack of effect on serum HDL cholesterol levels from the current study is in line with most other studies.[2]

In summary, in the absence of GH, transdermal testosterone stimulates whole body fat oxidation. Oral testosterone in the doses used did not significantly change fat oxidation. We cannot exclude a small hepatic effect, undetectable by the current technique. Although liver is a major site of lipid metabolism and a sex-steroid responsive organ, we conclude that the effect of testosterone on fat oxidation occurs predominantly in the periphery, rather than in the liver.

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Competing interests/financial disclosure

Nothing to declare.

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